THE UNIVERSITY OF ALBERTA

CENTRAL BIOGENIC AMINES AND THERMOREGULATION IN A HIBERNATOR, *SPERMOPHILUS RICHARDSONII*

by

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A THESIS

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ABSTRACT

The present study investigated the endogenous changes in thermoregulatory responses (heat production[HP], heat loss[HL], and body temperature[Tb]) to intracerebroventricular (i.c.v.) injections of 5-hydroxytryptamine (5-HT), noradrenaline (NA), and a monoamine oxidase inhibitor (harmaline) in a hibernator, the Richardson's ground squirrel (*Spermophilus richardsonii*) during different phases of the annual hibernation cycle. In the non-hibernating phase, at ambient temperature (Ta) = 5°C, 5-HT caused an initial activation of HL followed by a compensatory increase of HP, resulting in a decrease of Tb of 0.40°C. Harmaline caused an increase of HP with a slight increase of HL resulting in a maximal increase of Tb of 0.8°C. Noradrenaline caused an initial activation of HP which was followed by a compensatory increase of HL resulting in an increase of Tb of 0.21°C. In the hibernating phase at Ta = 5°C, 5-HT caused activation of HL with concurrent suppression of HP resulting in a drop of Tb of 1.65°C. Harmaline caused a marked decrease of HP with a slight decrease of HL resulting in a decrease of Tb of 0.74°C. Noradrenaline caused activation of HP with compensatory increase of HL resulting in an increase of Tb of 0.78°C. During rewarming from hibernation at Ta = 6°C, 5-HT caused initial suppression of HP and a greater overall HL which resulted in a slower rate of arousal as compared to controls. Noradrenaline caused a greater rate of HP which resulted in a faster rate of arousal as compared to controls.

It is concluded that the increase of brain 5-HT via either exogenous 5-HT administration or inhibition of endogenous 5-HT

degradation suppresses HP in the normothermic ground squirrels in cold in the hibernating phase. Exogenous 5-HT also suppresses HP during the initial stages of arousal from hibernation. Exogenous NA causes a greater increase of HP in the normothermic ground squirrels in the cold in the hibernating phase as compared to the non-hibernating phase. Exogenous NA also increased the rate of HP during arousal from hibernation. Since the animals were kept under constant photoperiod and temperature, the observed differential thermoregulatory responses (with respect to season of hibernation) following 5-HT, NA, and harmaline injections suggest an endogenous adjustment of thermoregulatory mechanisms incumbent with the circannual rhythm of hibernation.

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INTRODUCTION

That the mammalian hypothalamus contains relatively high concentrations of 5-hydroxytryptamine (5-HT; serotonin) and noradrenaline (NA) has been demonstrated by Amin *et al.* (1954), Vogt (1954) and Carlsson *et al.* (1962). In view of these findings, Brodie and Shore (1957) and von Euler (1961) hypothesized that these substances might be involved with the synaptic control of central thermoregulatory pathways. Early experiments by Feldberg and Myers (1963, 1964, and 1965) showed that 5-HT when injected centrally into the cat caused body temperature (Tb) to rise, while similar injections of adrenaline and NA caused Tb to fall. Subsequently, they hypothesized that Tb is controlled by a balance of catecholamines and 5-HT in the rostral hypothalamus.

As the scope of research broadened with the introduction of new injection techniques and utilization of new test species, the clear-cut role of biogenic amines in temperature regulation as put forward by Feldberg and Myers (1964) became controversial. For example, while central injections of 5-HT causes hyperthermia in cats (Feldberg and Myers, 1964), dogs (Feldberg *et al.*, 1966; 1967), and primates (Feldberg *et al*, 1967; Myers and Waller, 1973), similar injections cause hypothermia in sheep (Bligh *et al.*, 1971; Bacon and Bligh, 1976), rabbits (Cooper *et al.*, 1965; Bligh *et al.*, 1971), lambs (Cooper *et al.*, 1976), goats (Bligh *et al.*, 1971), oxen (Findlay and Thompson, 1968), mice (Brittain and Handley, 1967), and rats (Feldberg and Lotti, 1967; Myers and Yaksh, 1968). Likewise, while central injections of NA cause hypothermia in cats (Feldberg and Myers, 1964), dogs (Feldberg

et al., 1966), and primates (Feldberg *et al.*, 1967), similar injections cause hyperthermia in guinea pigs (Zeisberger and Brück, 1970, 1976), rabbits (Cooper *et al.*, 1965), rats (Beckman, 1970; Veale and Whishaw, 1976), and ground squirrels (Beckman and Satinoff, 1972; Glass and Wang, 1978). As yet, there is no concrete explanation for the species difference observed in response to central injections of biogenic amines. It has been suggested, however, that the brain of every species may be wired in an unique neurochemical manner (Cooper *et al.*, 1965). Also, it has been suggested that many of these differences may have arisen from contamination of injection fluid (Peindaries and Jacob, 1971), conducting experiments at unspecified environmental temperatures or using unspecified drug concentrations (Francesconi and Mager, 1976).

In spite of the inconsistencies in species response to central injections of biogenic amines, evidence from studies measuring changes in central biogenic amine levels and turnover rates in animals subjected to temperature stress have further substantiated the roles of these substances in mediating central thermoregulatory mechanisms. For example, in rats and mice where central 5-HT injection activates thermolytic mechanisms (Brittain and Handley, 1967), exposure to a warm environment causes increased 5-HT levels (Reid *et al.*, 1968; Harri and Turri, 1969), 5-HT synthesis (Corrodi *et al.*, 1967; Reid *et al.*, 1968), and 5-HT turnover (Simmonds, 1970). Exposure of these animals to a cold environment, on the other hand, causes decreased 5-HT levels (Harri and Turri, 1969) and a decrease in the activity of 5-HT neurons and 5-HT synthesis (Corrodi *et al.*, 1967). In cold ex-

posed monkeys in which central injection of 5-HT activates thermogenic mechanisms, an increase in 5-HT output was observed in the perfusate of the push-pull cannula which was designed to perfuse specific sites in the hypothalamus (Myers and Beleslin, 1971). Furthermore, in rats and mice in which central injection of NA activates thermogenic mechanisms (Beckman, 1970; Veale and Whishaw, 1976), exposure to warm causes decreased NA levels (Harri and Turri, 1969), while exposure to cold increases NA levels (Corrodi *et al.*, 1967; Simmonds, 1969), and turnover (Reid *et al.*, 1968). In the cat in which central injection of NA activates thermolytic mechanisms (Feldberg and Myers, 1964), exposure to warm causes an increase of NA output in the perfusate from a push-pull cannula situated in the hypothalamus (Myers and Chinn, 1973).

Additional support for biogenic amines acting as mediators of central thermoregulatory mechanisms comes from studies of the thermoregulatory effects of specific biogenic amine agonists and antagonists. Injections of the monoamine oxidase (MAO) inhibitors tranylcypromine and harmaline which increase brain 5-HT levels (Feldberg and Lotti, 1967; Brunivels and Sourkes, 1968) mimic the thermoregulatory effects of central injection of 5-HT. For example, in the rat, where central injections of 5-HT cause a drop in Tb (Myers and Yaksh, 1968), tranylcypromine (Feldberg and Lotti, 1967) and harmaline (Brunivels and Sourkes, 1968) also cause lowered Tb. Short-term increases in central 5-HT facilitated by i.p. injections of L-tryptophan and para-chlorophenylalanine (p-CPA) in the golden hamster (Janský *et al.*, 1973) have also been observed to cause lowered Tb.

In cats where central injection of 5-HT causes a rise in Tb, tranylcypromine has the same effect (Feldberg and Lotti, 1967). Agents such as phentolamine which inhibit the thermoregulatory effects of central injection of NA by blocking central α-receptors (Dhawan and Dua, 1971; Burks, 1972) are shown to have effects opposite to NA when injected by themselves. Furthermore, blockage of central NA synthesis by i.p. injection of D,L-alpha-methyl(-P-tyrosine methyl ester) (α-MpTme) in the cold causes hypothermia in cold-acclimated rats (Feist, 1970). Central injections of NA agonists such as the sympathomimetics naphazoline and tetrahydrozoline (Lomax and Foster, 1969), and phenylephrine (Rudy and Wolf, 1971), in rats, on the other hand, produce hyperthermia as does NA.

It is now evident that in any study concerned with the effects of central injection of putative neurotransmitters, stringent experimental regimes must be applied to properly assess the physiological significance of responses evoked by such injections. Such considerations have prompted several investigators to recommend the following criteria to be fulfilled prior to the evaluation of thermoregulatory responses achieved by exogenous administration of putative neurotransmitters (Myers, 1974; Francesconi and Mager, 1976): 1) the establishment of dose-response curves; 2) the monitoring of Tb for extended periods after drug administration; 3) the utilization of only sterile, non-pyrogenic media and equipment, and 4) the utilization of specific agonists or antagonists.

Recently, the central metabolism of 5-HT and NA has been found to undergo cyclic changes associated with the yearly hibernation cycle.

For example, profound increases in brain 5-HT concentration have been observed in the golden hamster (Novotná *et al.*, 1975), the bat (Shaskan, 1969), the hedgehog (Uuspaä, 1963), and the ground squirrel (Spafford and Pengelley, 1971). Increases in central 5-HT turnover during the preparative and maintenance phases of the annual hibernation cycle have also been observed in the golden hamster (Novotná *et al.*, 1975). According to Novotná and Janský (1975) the increase in central 5-HT turnover is not related to its destruction rates as they found no concurrent changes in MAO activity. Rather, they suggested that the increased 5-HT turnover was due to increased production of 5-HT in the brain due to an increased dietary intake of tryptophan, a 5-HT precursor, and the lowering of liver pyrrolase, an enzyme which decreases blood tryptophan levels. Recently, however, Voitenko (1977) has shown that increased brain 5-HT turnover may be associated with decreases in MAO activity during hibernation in the European hamster.

Occurring simultaneously with the increases of 5-HT metabolism are pronounced decreases in brain NA metabolism in the Arctic ground squirrel (Feist and Galster, 1974), the thirteen-lined ground squirrel (Draskóczy and Lyman, 1967), and the hedgehog (Uuspaä, 1963; Sauerbier and Lemmer, 1977). Also, brain turnover rates of NA have been observed to drop to very low levels during hibernation in the thirteen-lined ground squirrel (Draskóczy and Lyman, 1967) and in the hedgehog (Sauerbier and Lemmer, 1977). Furthermore, Faure and Calas (1977) have shown that the *in vitro* uptake of NA by central catecholaminergic fibers is reduced in brain slices taken from the hibernating hedgehog

as compared to that in brain slices taken from active hedgehogs.

The maintenance of brain levels of 5-HT and NA have been shown to be necessary for various phases of the hibernation cycle. Spafford and Pengelley (1971) demonstrated that by lowering brain levels of 5-HT by i.p. administration of p-CPA, or by lesioning the medial raphe nucleus to reduce endogenous synthesis of 5-HT, hibernation was terminated prematurely. In addition, Janský (1978) has shown that by feeding golden hamsters a tryptophan-rich diet, a maneuver aimed to increase the precursor for central 5-HT synthesis, the length of hibernation bouts was increased. Spafford and Pengelley (1971) showed that female golden mantled ground squirrels which have higher brain 5-HT levels than the males hibernate "better" in both duration and frequency of hibernation than the males. Also, the need for adequate NA levels during the arousal phase has been demonstrated by Feist (1970) who showed that the inhibition of central NA concentration by i.p. administration of α-MpTme prevents complete arousal from hibernation.

Thus, there is a great deal of evidence suggesting that 5-HT and NA are functionally involved with hibernation in mammals. To date, however, the nature of the roles played by these substances in governing the entrance into, the maintenance of, and the arousal from hibernation is open to speculation. Novotná and Janský (1976) and Janský (1978) have suggested that the increases in 5-HT metabolism occurring during the hibernating phase of the annual hibernating cycle may serve to inhibit the pituitary-adrenal axis, reducing the activities of some endocrine glands. On the other hand, some authors have suggested that 5-HT and NA may be involved with the thermoregulation of hibernation

(Feist and Galster, 1974).

In seasonal hibernators (e.g. ground squirrels and marmots), many physiological changes take place in preparation for hibernation during late summer. Typical changes signaling the beginning of the hibernating phase include a rapid weight gain (Pengelley and Fisher, 1963) and a lowering of oxygen consumption (Armitage and Shulenberger, 1972) and Tb (Scott *et al.*, 1974). When placed in the cold these animals will usually enter hibernation. In contrast, if the animal is in its non-hibernating phase, exposure to cold evokes thermoregulatory responses typical of those observed in the non-hibernating homeotherms rather than the exhibition of hibernation (Wang, unpublished). Thus, it is apparent that the hibernating and the non-hibernating phases represent two distinct physiological states.

The objective of this study, therefore, was to determine if there is differential thermoregulatory response to perturbation of brain 5-HT and NA levels in a seasonal hibernator (the Richardson's ground squirrel, *Spermophilus richardsonii*) during different phases of the annual hibernating cycle. Such information would shed more light on the possible role of central serotonergic and noradrenergic pathways in their participation of regulation of hibernation in seasonal hibernators.

MATERIALS AND METHODS

I. ANIMALS

Mature and juvenile Richardson's ground squirrels of both sexes were used in this study. These animals were live-trapped near Edmonton, Alberta and brought to the animal holding facilities at the University of Alberta. All animals were fed fresh vegetables, sunflower seeds, and Vitamite cubes (Northwest Feed, Edmonton) *ad libitum* and maintained under a 12L/12D photoperiod at an ambient temperature (Ta) of 23°C. Weekly measurements of weight were made to aid determination of endogenous phase for hibernation in each animal. The hibernation phase was further verified by exhibition of hibernation in cold (5°C) and dark without food in a walk-in environmental chamber after the injection experiment. The non-hibernating phase was evident when the animal showed no weekly weight increase and did not hibernate when placed in cold and dark without food for up to 12 days.

For arousal experiments, the ground squirrels were placed in the cold (6°C) and dark four weeks prior to experimentation. Fresh vegetables, sunflower seeds, and Vitamite cubes were supplied *ad libitum*. Animals were checked daily for hibernation using the sawdust technique, and were used for experimentation only if they had been hibernating for 2 consecutive days, with rectal temperatures of 6.5 - 7.5°C at time of experimentation.

II. SURGERY AND CANNULATION

Under sodium pentobarbital anaesthesia (Nembutal, 50mg/kg)

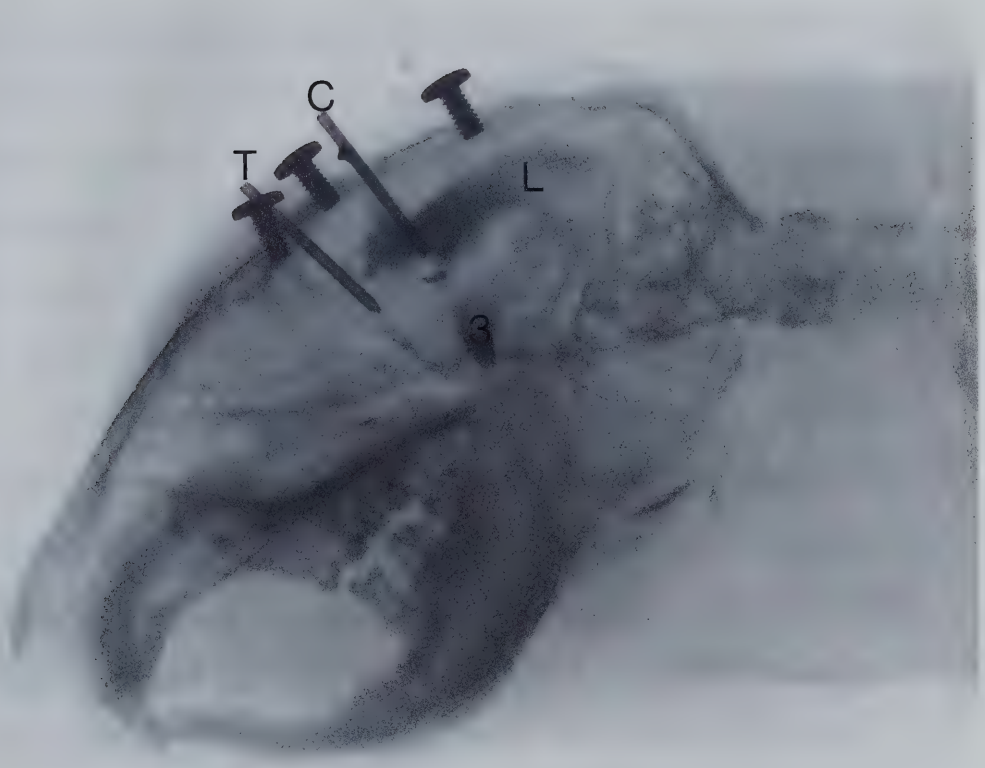
Fig. 1. X-ray ventriculograph showing the diffusion of a radio-opaque dye (90% hypaque solution, 10 µl) throughout the lateral and third ventricles 15 sec after injection through the cannula re-entry tube in an anaesthetised Richardson's ground squirrel. The thermocouple re-entry tube is located above the preoptic anterior hypothalamus. The 3 screws were used to anchor a protective acrylic cap (not shown) to the skull.

C = cannula re-entry tube

L = lateral cerebral ventricle

T = thermocouple re-entry tube

3 = third ventricle


C
T
L
3

supplemented with Ketaset (25mg/kg, Rogar), each animal was stereotaxically implanted with a chronic cannula guide tube in the left lateral cerebral ventricle. The final stereotaxic co-ordinates (AP 3.9, L 2.3, H 4.0[using earbars as zero point]) were determined by histological sectioning and eosin dye injection. After experimentation, the position of the cannula guide tube was further verified by X-ray analysis of the diffusion of a radio-opaque dye (90% Hypaque solution) throughout the ventricular system (Fig. 1). The cannula guide tubes were fabricated from 22 gauge stainless-steel hypodermic tubing and contained an indwelling stylus made from 28 gauge stainless-steel wire. After implantation, a cap made of sealed polyethylene tubing (PE 50) was affixed to the guide tube to prevent leakage or contamination when not in use. The guide tube was then cemented in place with dental acrylic and anchored with stainless-steel screws screwed into the skull. A plastic ring shield made from the cap of a 1 cc disposable syringe was then embedded into the acrylic for protection of the guide tube. Animals were also implanted with a thermocouple re-entry tube made from 22 gauge stainless-steel tubing. These were implanted above the preoptic hypothalamic region (Fig. 1).

For injection, the tip of a 10 µl Hamilton syringe needle was machined to fit snugly within the 22 gauge guide tube with its tip extending 0.5 mm into the ventricular lumen. This truncated needle was connected to a 10 µl Hamilton syringe by a 2 m length of PE 10 tubing which facilitated remote injection. Before injection, syringe, tubing, and needle were repeatedly flushed with 70% ethanol, sterile saline, and finally, injection fluid. Between experiments these were

stored in 70% ethanol.

III. DRUGS

Immediately before injection, solutions of 5-HT creatinine sulphate (Sigma), harmaline HCl (Sigma), or L-arterenol bitartrate (NA) (Sigma), each in concentration of 10 μg/μl were prepared using sterile artificial cerebrospinal fluid (Myers and Buckman, 1972) which had been passed through a .22 μm bacterial filter (Millipore). This fluid was thought to have been pyrogen-free because no febrile response was ever observed after central injection of artificial cerebrospinal fluid alone. For serial injections of 5-HT, 10, 5, 2.5, and 1 μl were injected over 30 sec delivering doses of 56, 28, 14, and 6 μg of 5-HT (free base), respectively. For control injection, equimolar solutions of creatinine sulphate (Sigma) were injected at the same volumes as 5-HT injections. For harmaline injections, 20 μl was injected, delivering a dose of 170 μg harmaline. For serial injections of NA, 1, 1.5, and 2.5 μl were injected over 30 sec delivering doses of 5, 7.5, and 12.5 μg of NA (free base), respectively. In arousal experiments, drugs were prepared and injected in a similar manner except that 3 single injections of 5-HT or NA were given when brain temperature (Tbr) reached 10, 20, and 30^{o}C. The total amount of 5-HT administered during an arousal was 168 μg, and for NA, 37.5 μg.

IV. OXYGEN CONSUMPTION

Air supply to the animal was from the compressed air line in the laboratory. It was first decompressed to 5.5 psi by a low pressure regulator (Model 70, Matheson), and then dried by passing

through a tube containing Drierite (Fisher Scientific Co.). Flowrate (1500 cc/min STP) to the animals was metered by individually calibrated flow meters (Model 603, Matheson) before it reached the animal chamber. The animal chamber was held within a temperature controlled cabinet which maintained the Ta inside the animal chamber at either 23 or 5 $\pm$ 0.5^{o}C. Exhaust from the animal chamber was passed through a tube containing Ascarite (A.A. Thomas Co.) and Drierite for CO_2 and water vapor removal before it was measured by a Beckman G-2 paramagnetic oxygen analyser. The output from the analyser was recorded by a Honeywell strip chart recorder, and synchronously computed and integrated at once per sec by a Texas Instruments digital computer (Wang and Peter, 1975). The 90% equilibration time constant of the measuring system was 10 min.

V. HEAT LOSS

Dry heat loss(HL) of animals was measured simultaneously with oxygen consumption using a Thermonetics (model SECA-1201) gradient-layer calorimeter. The output of which was fed to a strip chart recorder (Model #282, Linear), and into the computer for calculation of instantaneous rate of HL and integrated total HL at once per sec. The calorimeter also served as the metabolism chamber. Temperature of the calorimeter was regulated at either 23 or 5 $\pm$ 0.1^{o}C by the circulation of coolant through the walls of the calorimeter from a constant temperature water bath. The 90% equilibration time constant of the calorimeter was 12 min.

VI. BODY TEMPERATURE MEASUREMENTS

Core temperature (Tb; Tre) was measured by inserting a copper-constantan thermocouple made from 30 guage wires sheathed by PE 50 tubing 6 cm into the rectum. In arousal experiments, Tbr was measured by inserting a copper constantan thermocouple made from 40 guage wires into the brain thermocouple re-entry tube. Temperatures were measured with a resolution of 0.01^{o}C accuracy with a digital voltmeter and by comparison to a reference voltage using a custom soft-ware program in the computer.

VII. EXPERIMENTAL PROTOCAL

Where injections into normothermic ground squirrels were made, the animal was restrained in a custom-made wire meshed cylinder with the head extruding beyond the cylinder. This procedure was necessary to prevent chewing of the cannula by the animal and eliminated the behavioral component for variations of HL and heat production (HP) due to random movements of the animal. Thus, changes observed in these experiments represented definitive physiological adjustments independent of behavioral changes. All drugs were injected remotely via a 2 m extension of cannula (PE 10) and i.c.v. in a single injection over 30 sec.

In experiments concerned with arousal from hibernation, the hibernating animal was immediately transferred from the hibernaculum to the precooled gradient-layer calorimeter, where it was fitted with brain and rectal thermocouples and injection cannula. Insertion of the rectal thermocouple, was in most cases, sufficient stimulus to

evoke arousal from hibernation. All drugs were injected remotely via a 2 m extension of PE 10 tubing and i.c.v. In preliminary experiments, control injections of artificial cerebrospinal fluid alone or with creatinine sulphate had no effect on thermoregulation of normothermic or arousing ground squirrels. Therefore, no control injections were administered in the arousal experiments.

Where applicable, the assessment of thermoregulatory responses achieved by exogenous administration of putative neurotransmitters was carried out under conditions recommended by Francesconi and Mager (1976).

VIII. CALCULATIONS

Drug induced changes in HP and HL in experiments on normothermic animals were calculated by taking the difference between the integrated totals (at an integration rate of once per sec) of HP and HL of equal times before and after the injection. Values for preinjection were based on recordings obtained 15 min immediately preceding the injection. Multiples of preinjection values were used when post-injection response lasted more than 15 min. Average changes in Tb due to drug action were calculated by averaging Tb measurements at 5 min intervals throughout the duration of response and subtracting this from the averaged Tb observed during the 15 min pre-injection period. In all experiments, oxygen consumption was converted to HP by using a caloric equivalence of 5 cal/ccO_2, assuming an RQ of 0.90 (Kleiber, 1961). Heat deficit or gain was calculated by subtracting the net HL from net HP after drug injection. Heat production and HL values were expressed as cal/Wt (in grams)$^{0.75}$.

IX. STATISTICS

All data analysis was carried out by using an unpaired student's t-test. (Sokal and Rohlf, 1969). The level for significance was set at $p<0.05$.

RESULTS

I. NORMOTHERMIC ANIMALS

A. 5-HT

Figure 2 shows the time-course of thermoregulatory response of two Richardson's ground squirrels (#D-10, non-hibernating phase, #404, hibernating phase) to 28 μg 5-HT i.c.v. In the non-hibernating phase, 5-HT caused a rapid increase of HL reaching a maximum of 135 cal/15 min, 15 min after injection. This resulted in an initial $0.4^{o}C$ drop in Tb. A compensatory increase in HP occurred 15 min after the injection which reached a peak value of 42.0 $cc O_2$/15 min (210 cal/15 min) 30 min after the injection. During the hibernating phase, 5-HT caused an increase of HL reaching a maximum of 180 cal/15 min, 15 min after injection. There was a simultaneous suppression of HP which showed a maximum depression of 59.0 $cc O_2$/15 min (295 cal/15 min) 15 min after injection. This resulted in a drop of Tb of $1.0^{o}C$. All parameters returned to preinjection levels approximately 45 min after injection.

Figure 3 shows the dose-response relationship of Richardson's ground squirrels in the non-hibernating phase (n=4) and in the hibernating phase (n=4) to 4 serial injections (6, 14, 28, and 56 μg) of 5-HT. Throughout the dose range of 5-HT injected (6-56 μg), ground squirrels in their non-hibernating phase increased their HP from $.38 \pm 0.57$ to 2.38 ± 0.70 cal/$Wt^{0.75}$, and their HL from 0.87 ± 0.62 to 3.75 ± 0.78 cal/$Wt^{0.75}$. These changes resulted in increases of heat deficit of 0.15 ± 0.14 to 2.42 ± 0.50 cal/$Wt^{0.75}$ and were reflected by changes in Tb from $^{+}0.10 \pm 0.10$ to $^{-}0.40 \pm 0.07^{o}C$. In contrast, ground squirrels in their hibernating phase showed decreases of HP from 1.73 ± 0.40 to

Fig. 2. The time-course of thermoregulatory response of two Richardson's ground squirrels (#D-10, non-hibernating phase; #404, hibernating phase) to 28 μg 5-HT (i.c.v.) at Ta=5°C. Top; change in heat loss by 5-HT injection. Middle: change in oxygen consumption by 5-HT injection. Bottom: change in rectal temperature by 5-HT injection. Positive and negative values indicate activation and suppression, respectively, of a parameter in comparison to its pre-injection values.

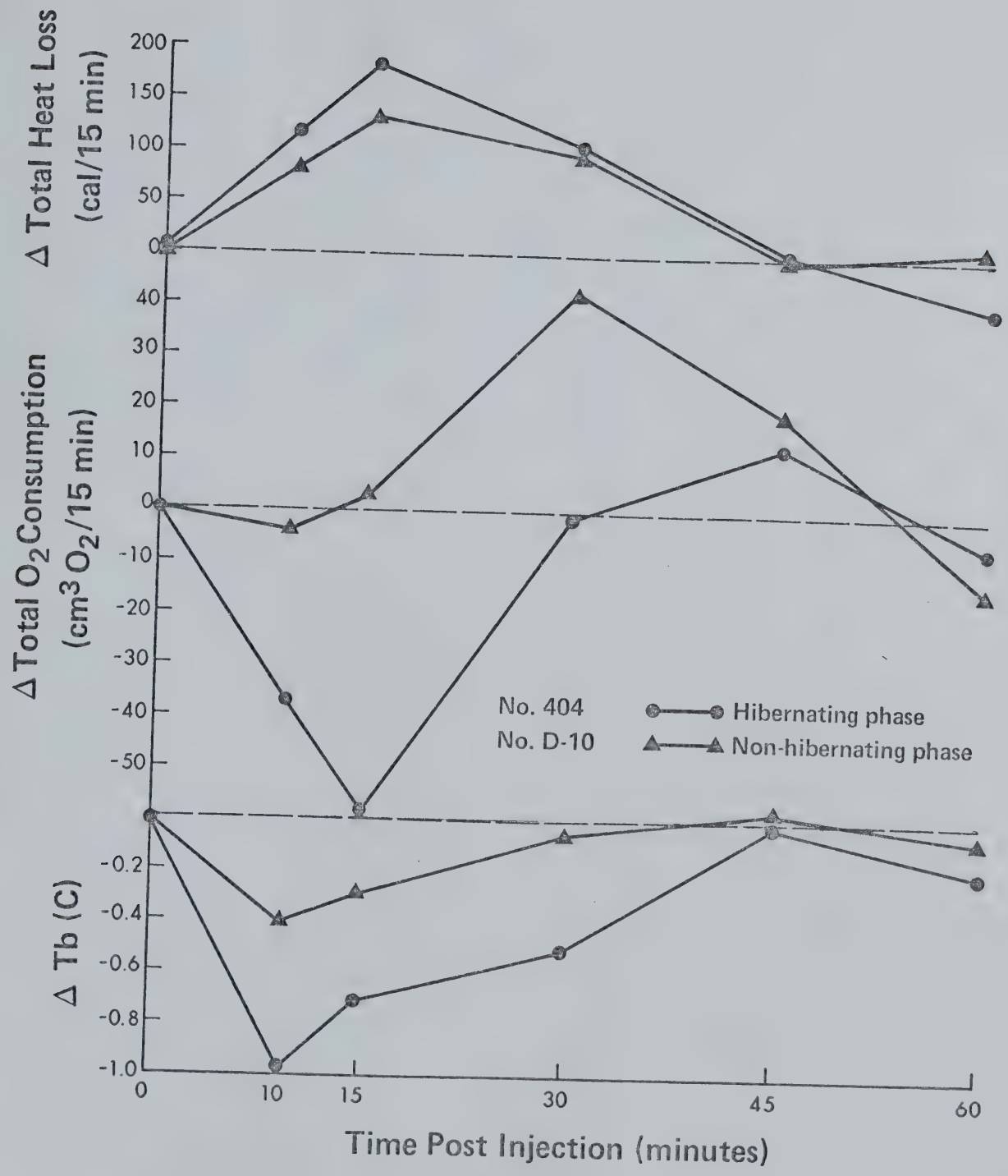
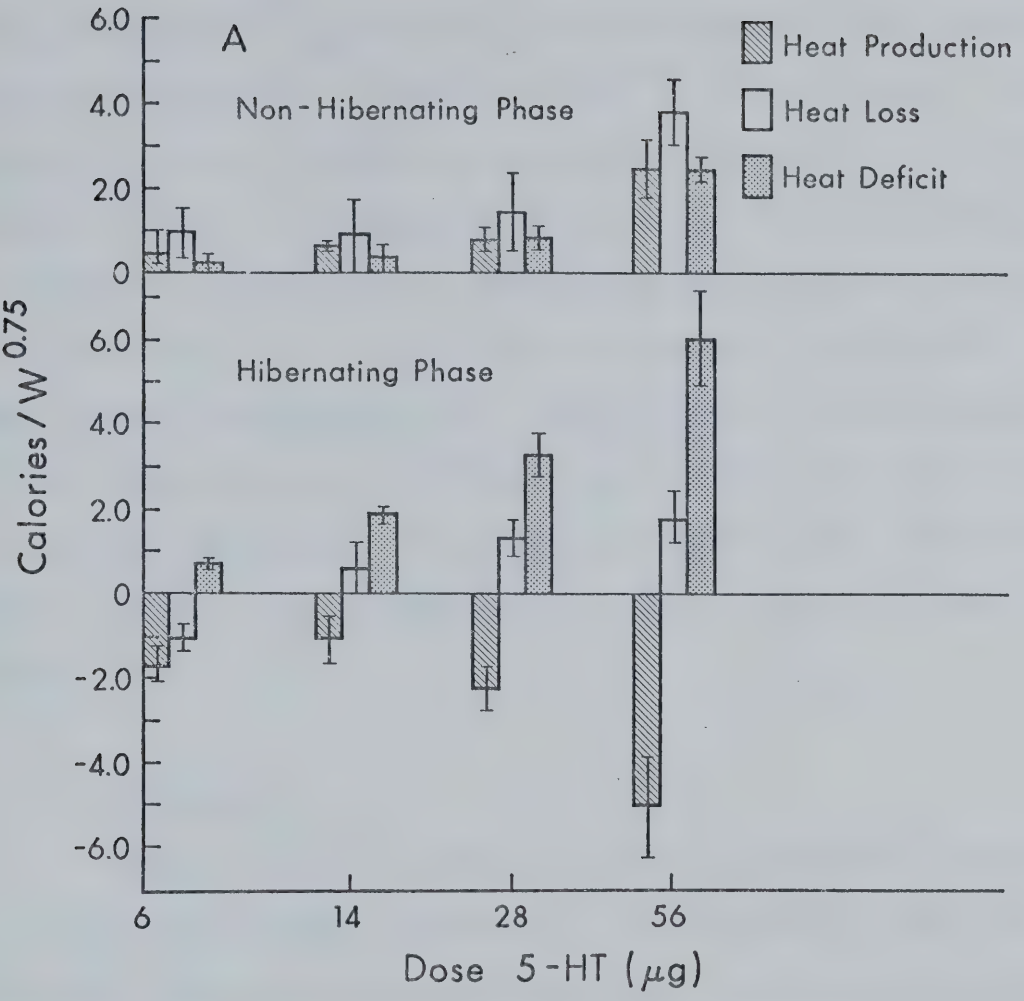
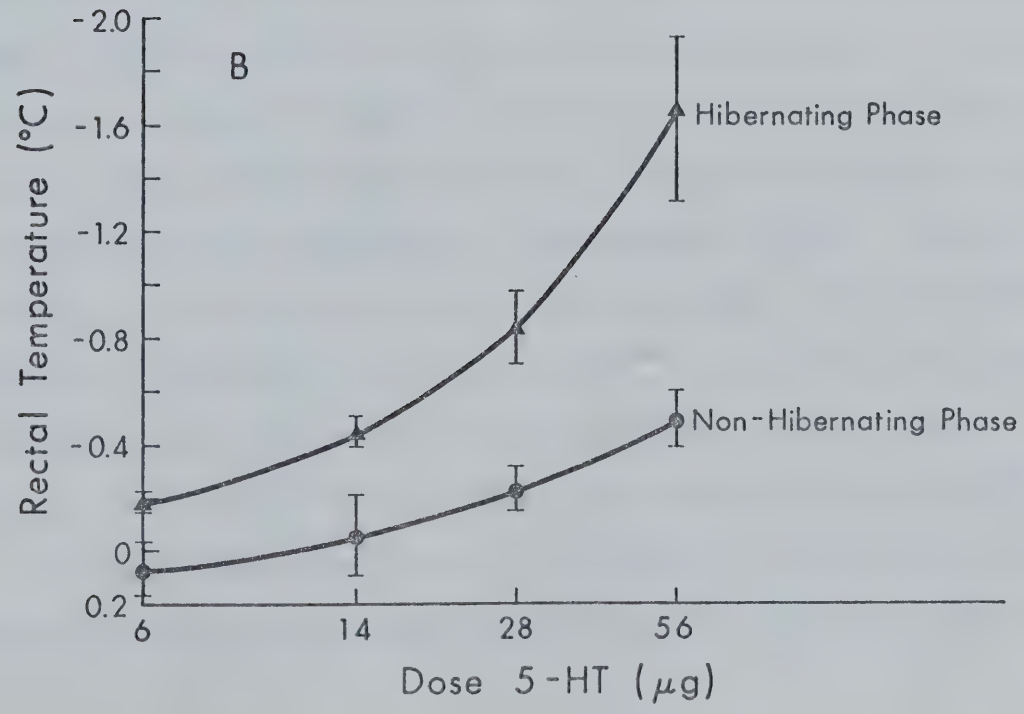

Δ Total Heat Loss (cal/15 min)
200
150
100
50
0
Δ Total O_2 Consumption (cm^3 O_2/15 min)
40
30
20
10
0
-10
-20
-30
-40
-50
No. 404
No. D-10
Hibernating phase
Non-hibernating phase
Δ Tb (C)
-0.2
-0.4
-0.6
-0.8
-1.0
0
10
15
30
45
60
Time Post Injection (minutes)

Fig. 3. The dose-response relationship of Richardson's ground squirrels in the non-hibernating phase (n=4) and in the hibernating phase (n=4) to 4 serial injections (6, 14, 28, and 56 µg) of 5-HT (i.c.v.) at Ta = 5°C. A: (top) heat production, heat loss and heat deficit elicited by 5-HT in the non-hibernating phase. (bottom) heat production, heat loss and heat deficit elicited by 5-HT in the hibernating phase. B: changes in rectal temperature over the same dose-range of 5-HT during the non-hibernating and hibernating phases. Bars are S.E.M. Positive and negative values indicate activation and suppression, respectively, of a parameter in comparison to its pre-injection values.


A
Heat Production
Heat Loss
Heat Deficit
Non-Hibernating Phase
Hibernating Phase
Calories / W 0.75
6.0
4.0
2.0
0
-2.0
-4.0
-6.0
6
14
28
56
Dose 5-HT (μg)


B
Hibernating Phase
Non-Hibernating Phase
Rectal Temperature (°C)
-2.0
-1.6
-1.2
-0.8
-0.4
0
0.2
6
14
28
56
Dose 5-HT (μg)

5.18±1.33 cal/$Wt^{0.75}$, and decrease of HL of 0.96±0.38 at the lowest dose but increases up to 1.26±0.66 cal/$Wt^{0.75}$ at higher doses. These changes resulted in increases of heat deficit of 0.77±0.10 to 6.00±1.24 cal/$Wt^{0.75}$, and were reflected by decreases in Tb from 0.19±0.02 to 1.65±0.25°C. Comparing the non-hibernating and hibernating phases, the changes of HP by 5-HT were significantly different ($p<.05$) at all doses, as were the changes of Tb. The magnitude of HL was comparable at lower doses, but was significantly less ($p<.05$) at the highest dose in the hibernating phase. The overall heat deficit was significantly greater ($p<.05$) in the hibernating phase for all doses except the lowest.

B. HARMALINE

Figure 4 shows the time-course of thermoregulatory response of 2 Richardson's ground squirrels (#17, non-hibernating phase; #32, hibernating phase) to 170 µg harmaline i.c.v. In the non-hibernating phase, harmaline caused a rapid, initial increase of HL followed by a decrease and moderate increase. The net increase of HL was 78.0 cal for the entire 60 min after injection. The oxygen consumption showed an initial decrease followed by a compensatory increase. The net increase in HP was 166 cal for the 60 min duration. The excess HP caused an increase of Tb averating 0.30°C. In the hibernating phase, harmaline caused a net decrease of HL of 30 cal in 50 min and a decrease of HP of 350 cal resulting in an average Tb fall of 0.50°C.

Figure 5 shows the response of Richardson's ground squirrels in the non-hibernating phase (n=4) and hibernating phase (n=5) to i.c.v.

Fig. 4. The time course of thermoregulatory response of two Richardson's ground squirrels (#17, non-hibernating phase; #32, hibernating phase) to 170 μg harmaline (i.c.v.) at Ta = 5°C. Top: change in heat loss by harmaline injection. Middle: change in oxygen consumption by harmaline injection. Bottom: change in rectal temperature by harmaline injection. Positive and negative values indicate activation and suppression, respectively, of a parameter in comparison to its pre-injection values.

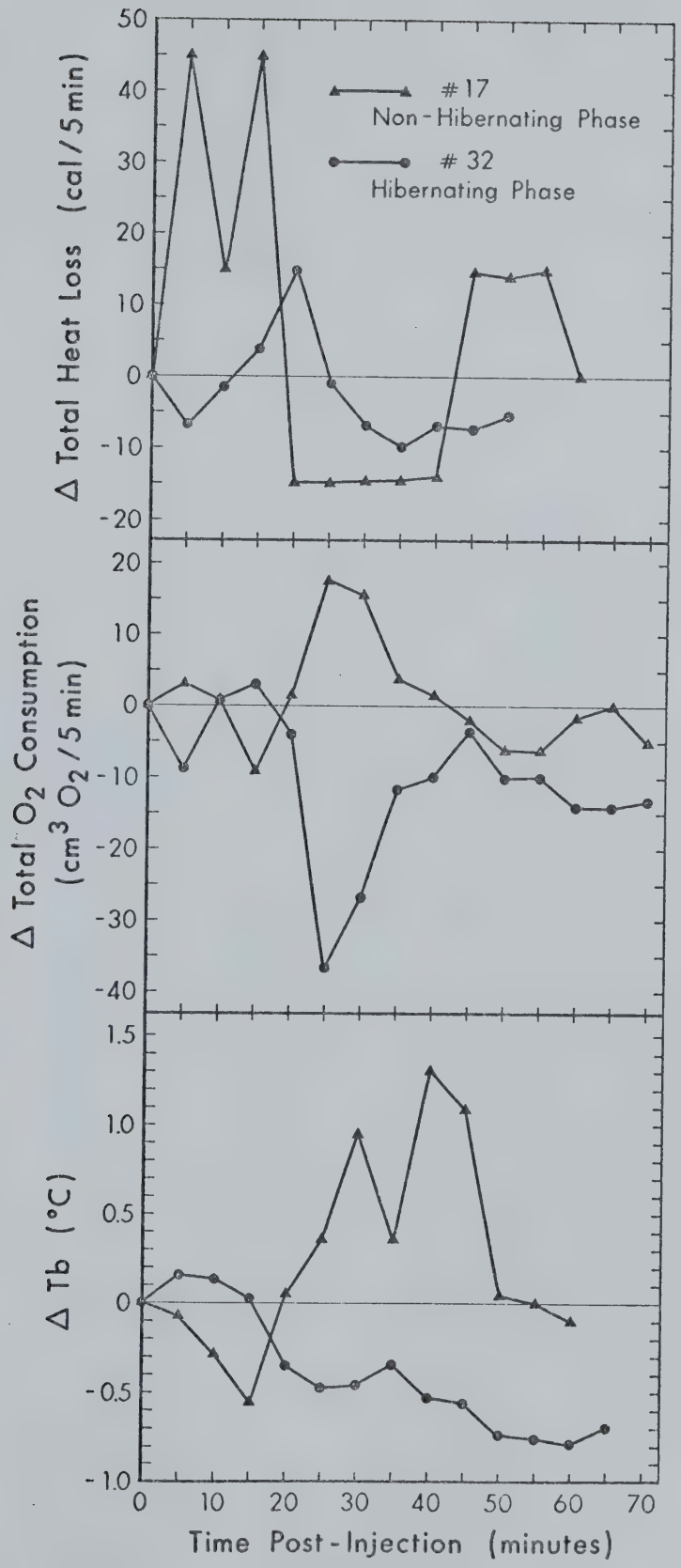
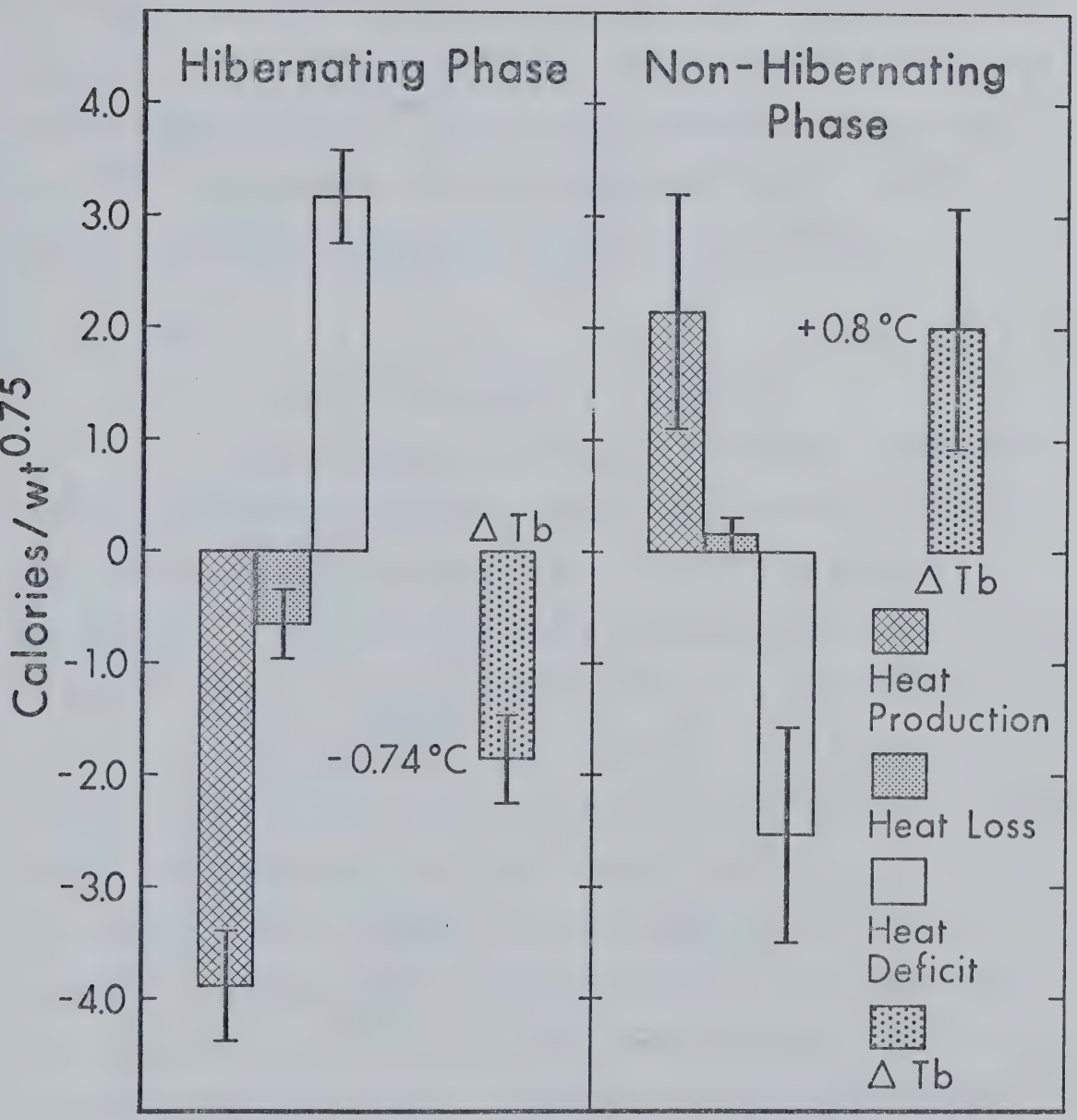

#17
Non-Hibernating Phase
32
Hibernating Phase
Δ Total Heat Loss (cal/5 min)
Δ Total O_2 Consumption (cm^3 O_2/5 min)
Δ Tb (°C)
Time Post-Injection (minutes)

Fig. 5. The response of Richardson's ground squirrels in the non-hibernating phase (n=4) and hibernating phase (n=5) to i.c.v. injection of harmaline at Ta = 5°C. Right: changes in heat production, heat loss, heat deficit and rectal temperature in the non-hibernating phase. Left: changes in heat production, heat loss, heat deficit and rectal temperature in the hibernating phase. Bars are S.E.M. Positive and negative values indicate activation and suppression, respectively, of a parameter in comparison to its pre-injection values.


Hibernating Phase
Non-Hibernating Phase
Calories / wt^0.75
4.0
3.0
2.0
1.0
0
-1.0
-2.0
-3.0
-4.0
Δ Tb
-0.74 °C
+0.8 °C
Δ Tb
Heat Production
Heat Loss
Heat Deficit
Δ Tb

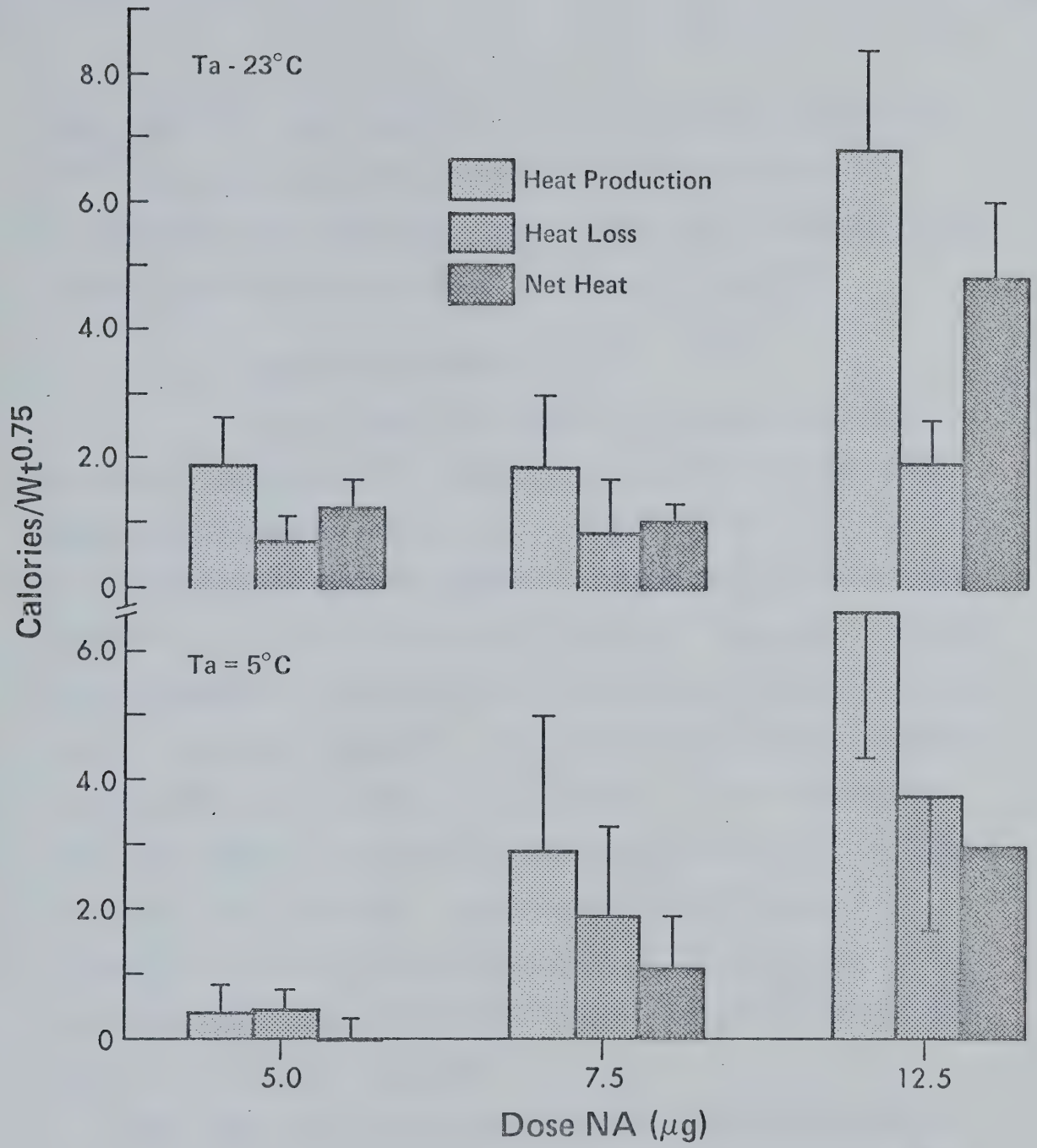
injection of 170 μg harmaline. In the non-hibernating phase, harmaline caused an increase of HP of 2.15±1.01 cal/$Wt^{0.75}$, and a slight increase of HL of 0.12±0.07 cal/$Wt^{0.75}$. This resulted in a heat gain of 2.54±1.00 cal/$Wt^{0.75}$ and was reflected by a mean increase in Tb of 0.80±0.24°C. In the hibernating phase, harmaline caused a suppression of HPof 3.86±0.49 cal/$Wt^{0.75}$, and a slight decrease of HL of 0.62±0.27 cal/$Wt^{0.75}$. This resulted in a heat deficit of 3.18±0.37 cal/$Wt^{0.75}$, and was reflected by a mean decrease in Tb of 0.74±0.08°C.

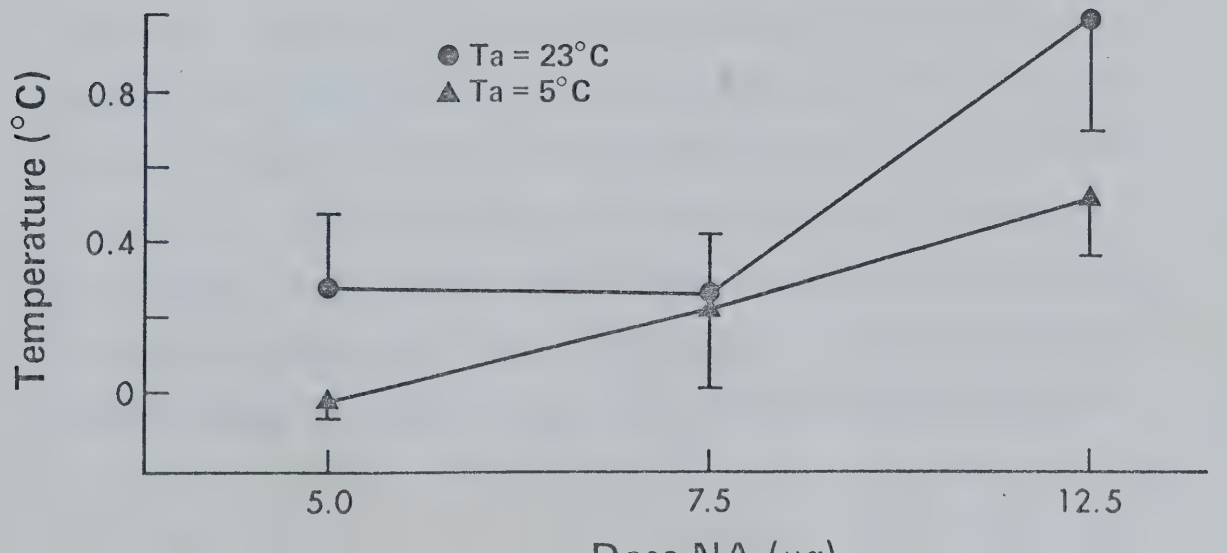
C. NA

1. Effects of Environmental Temperature

Figure 6 shows the effects of environmental temperature on the thermoregulatory response of Richardson's ground squirrels in their hibernating phase to serial i.c.v. injections of NA (5.0, 7.5, and 12.5 μg). In the warm (23°C [n=4]) the lowest dose (5.0 μg) caused an increase of HP of 1.91±0.78 cal/$Wt^{0.75}$ ($\bar{X}$ ± S.E.) and an increase of HL of 0.71±0.42 cal/$Wt^{0.75}$. This resulted in a net heat gain of 1.21±0.47 cal/$Wt^{0.75}$ which was reflected by an increase of Tb of 0.29± 0.20°C. The highest dose (12.5 μg) caused an increase of HP of 6.82± 1.50 cal/$Wt^{0.75}$ and an increase of HL of 1.95±0.69 cal/$Wt^{0.75}$. This resulted in a net heat gain of 4.88±1.13 cal/$Wt^{0.75}$ which was reflected by an increase of Tb of 1.03±0.28°C. In the cold (5°C [n=4]) the lowest dose (5.0 μg) caused an increase of HP of 0.32±0.33 cal/$Wt^{0.75}$ and an increase of HL of 0.34±0.41 cal/$Wt^{0.75}$. This resulted in a net heat deficit of 0.02±0.35 cal/$Wt^{0.75}$ which was reflected by a decrease of Tb of 0.05±0.08°C. The highest dose (12.5 μg) caused an increase of HP of 6.61±2.29 cal/$Wt^{0.75}$ and an increase of HL of 3.77±

Fig. 6. The dose-response relationship of Richardson's ground squirrels in their hibernating phase in the warm (23°C [n=4]), and in the cold (5°C [n=4]) to 3 serial injections (5.0, 7.5, and 12.5 µg) of NA (i.c.v.). Top: changes in heat production, heat loss and net heat gain elicited by NA. Bottom: changes in rectal temperature elicited by NA. Bars are S.E.M.


Ta - 23°C
Heat Production
Heat Loss
Net Heat
Calories/Wt^0.75
8.0
6.0
4.0
2.0
0
Ta = 5°C
6.0
4.0
2.0
0
5.0
7.5
12.5
Dose NA (μg)


Ta = 23°C
Ta = 5°C
Temperature (°C)
0.8
0.4
0
5.0
7.5
12.5

2.30 cal/$Wt^{0.75}$. This resulted in a net heat gain of 2.84±0.29 cal/$Wt^{0.75}$ which was reflected by an increase of Tb of 0.53±0.13°C. Throughout the dose range, there was no significant difference ($p>.05$) in all measured parameters between injections at 23 or 5°C.

2. Seasonal Differences

Figure 7 shows the time-course of thermoregulatory response of two Richardson's ground squirrels (#10, non-hibernating phase; #5, hibernating phase) to 12.5 µg NA i.c.v. at Ta = 23°C. In the non-hibernating phase NA caused an increase of HP reaching a maximum of 115 cal/15 min, 30 min after injection. There was an initial decrease and then an increase of HL which reached a maximum of 90 cal/15 min, 45 min after injection. This resulted in a maximal increase of Tb of 0.41°C occurring 30 min after injection. In the hibernating phase, NA caused an increase of HP reaching a maximum of 300 cal/15 min, 30 min after injection. There was an initial decrease of HL followed by an increase reaching a maximum of 123 cal/15 min, 45 min after injection. This resulted in a maximal increase of Tb of 1.64°C occurring 45 min after injection.

Since there was no significant difference between the thermoregulatory responses of Richardson's ground squirrels at 23 or 5°C, the data from experiments at both temperatures were pooled and presented in Figure 8. Figure 8 shows the effects of serial injections of NA (5.0, 7.5, and 12.5 µg) on the thermoregulatory response of Richardson's ground squirrels in the non-hibernating phase (n=6) and in the hibernating phase (n=9). Throughout the dose range of NA injected, ground squirrels in their non-hibernating phase increased

Fig. 7. The time-course of thermoregulatory response of two Richardson's ground squirrels (#10, non-hibernating phase; #5, hibernating phase) to 12.5 μg NA (i.c.v.) at Ta = 5°C. Top: change in heat loss by NA injection. Middle: change in heat production by NA injection. Positive and negative values indicate activation and suppression, respectively, of a parameter in comparison to its pre-injection values.

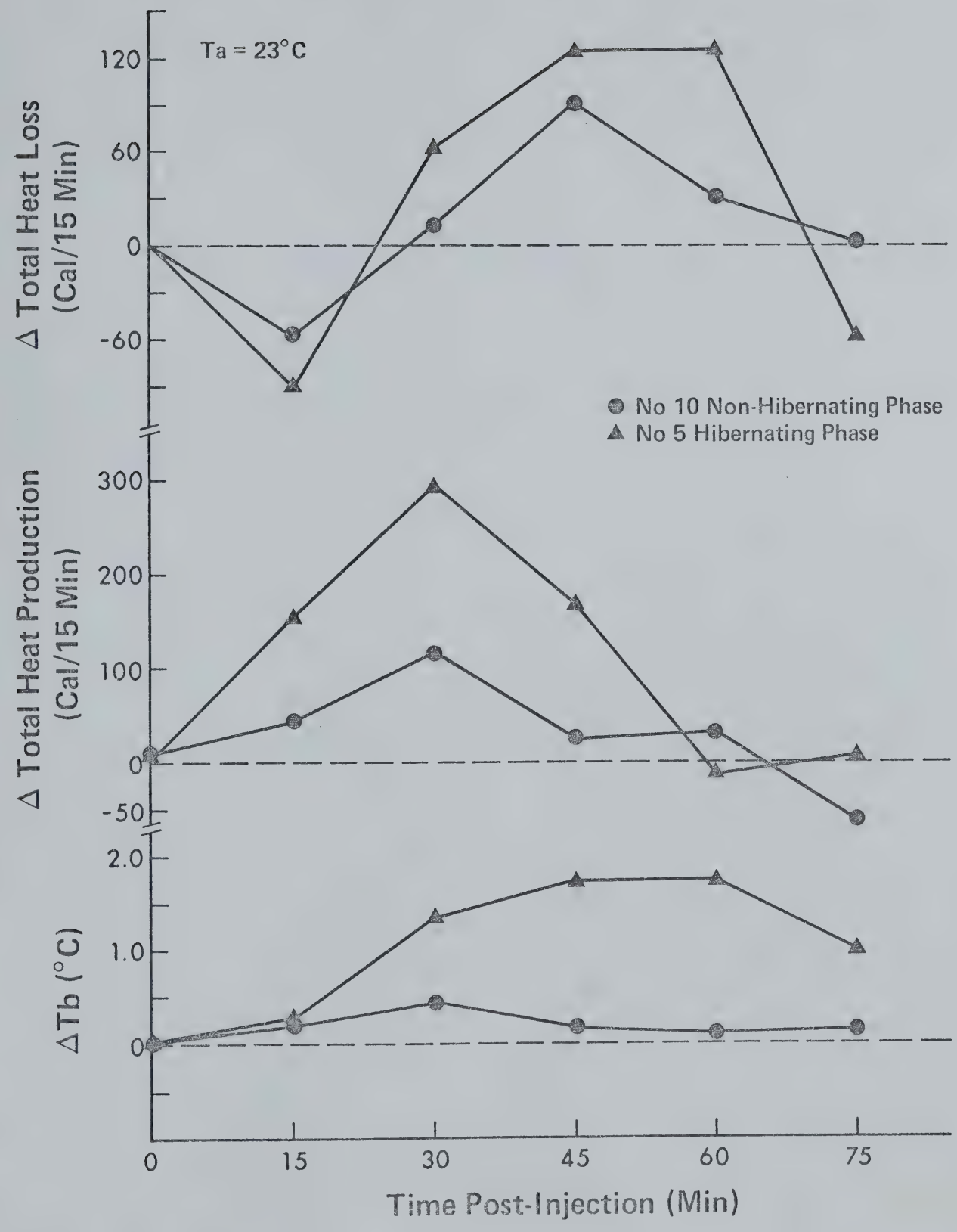
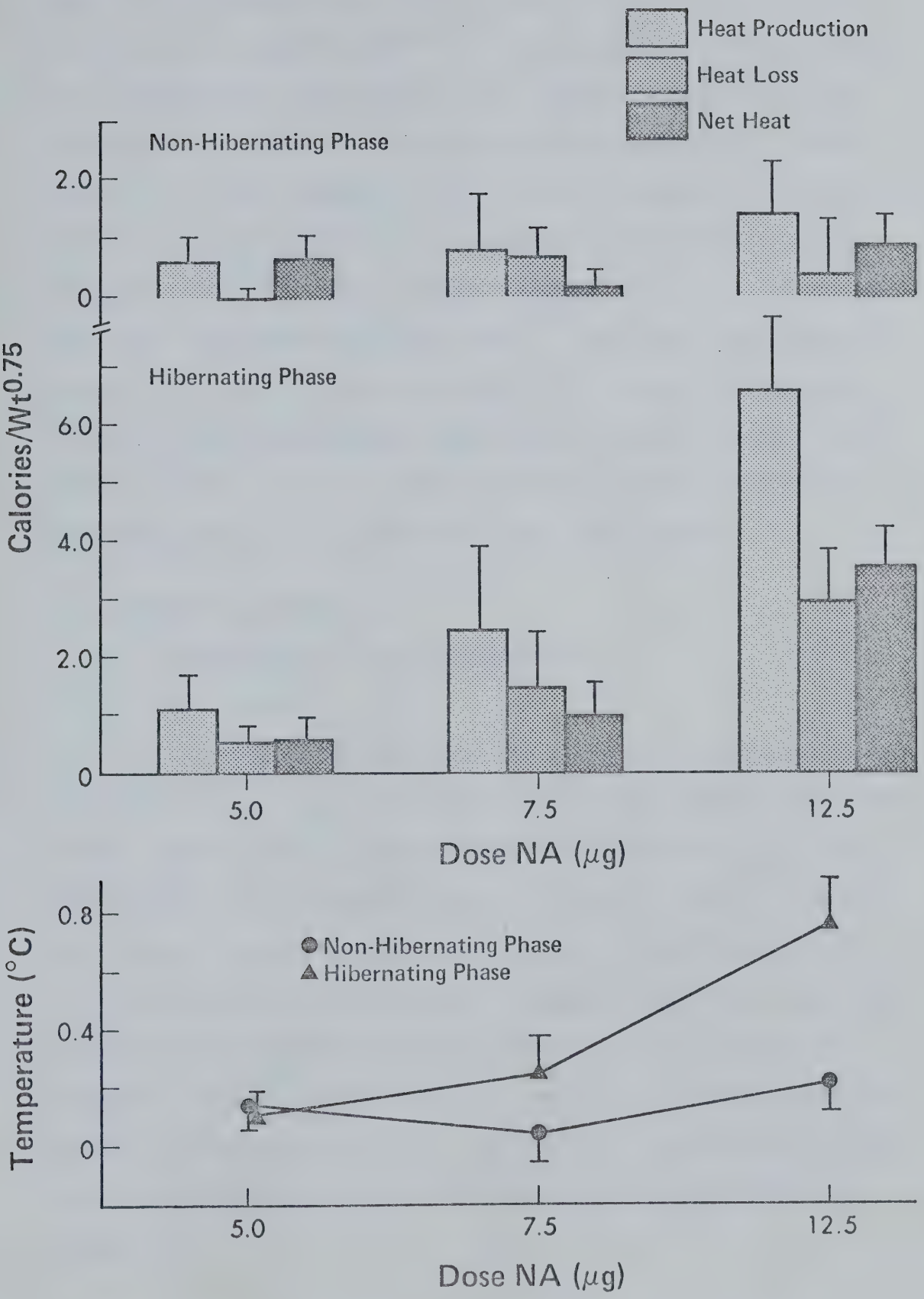

Ta = 23°C
Δ Total Heat Loss (Cal/15 Min)
120
60
0
-60
No 10 Non-Hibernating Phase
No 5 Hibernating Phase
Δ Total Heat Production (Cal/15 Min)
300
200
100
0
-50
ΔTb (°C)
2.0
1.0
0
0
15
30
45
60
75
Time Post-Injection (Min)

Fig. 8. The dose-response relationship of Richardson's ground squirrels in the non-hibernating phase (n=6) and in the hibernating phase (n=9) to 3 serial injections (5.0, 7.5, and 12.5 μg) of NA (i.c.v.) at Ta = 5^{o}C. Top: changes in heat production, heat loss and net heat gain elicited by NA. Bottom: changes in rectal temperature elicited by NA. Bars are S.E.M.


Heat Production
Heat Loss
Net Heat
Non-Hibernating Phase
Hibernating Phase
Calories/Wt0.75
2.0
0
6.0
4.0
2.0
0
5.0
7.5
12.5
Dose NA (μg)
Temperature (°C)
0.8
0.4
0
Non-Hibernating Phase
Hibernating Phase
5.0
7.5
12.5
Dose NA (μg)

their HP from 0.60±0.41 to 1.54±0.95 cal/$Wt^{0.75}$, and their HL from -0.03±0.15 to 0.51±0.93 cal/$Wt^{0.75}$. These changes resulted in increases of net heat gain from 0.27±0.36 to 1.03±0.51 cal/$Wt^{0.75}$ and were reflected by increases of Tb from 0.18±0.08 to 0.21±0.10°C. Ground squirrels in their hibernating phase showed increases in HP from 1.07± 0.57 to 6.71±1.28 cal/$Wt^{0.75}$, and increases in HL from 0.54±0.29 to 3.09±1.19 cal/$Wt^{0.75}$. These changes resulted in increases of net heat gain from 0.53±0.40 to 3.62±0.66 cal/$Wt^{0.75}$, and were reflected by increases in Tb from 0.16±0.09 to 0.87±0.16°C. Between the non-hibernating and hibernating phases, significantly greater increases in HP ($p<.02$) and net heat gain ($p<.01$) and Tb ($p<.02$) were found in the hibernating phase only at the highest dose (12.5 µg NA).

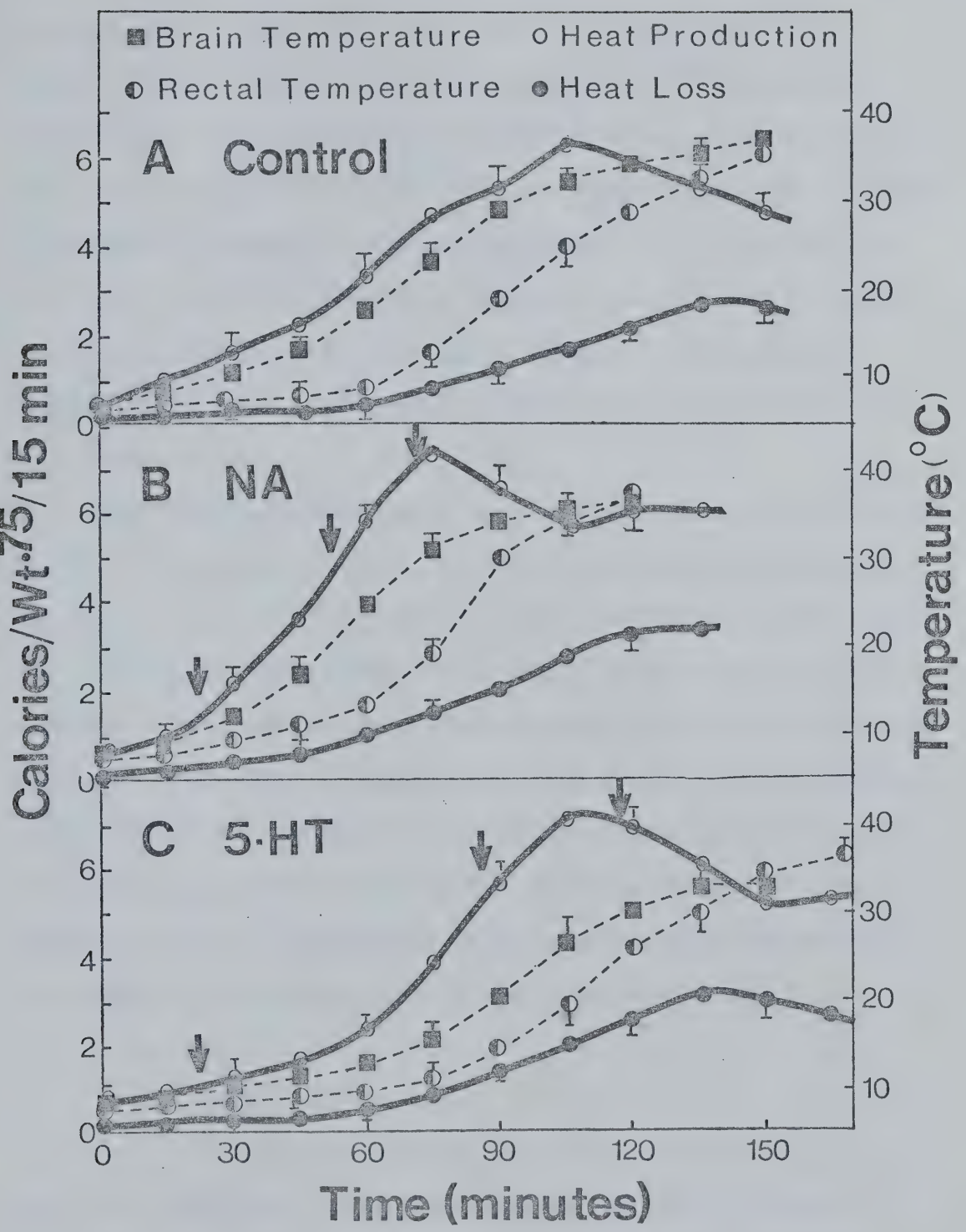
II. HIBERNATING ANIMALS

A. EFFECTS OF 5-HT AND NA ON AROUSAL

1. Control

Figure 9A illustrates the rates of HP and HL and changes in Tbr and Tre for the control group (n=7) during rewarming from hibernation. Starting from an initial level of 0.65±0.07 ($\bar{X}$ ± S.E.) cal/$Wt^{0.75}$/15 min, HP increased to a peak of 6.31±0.31 cal/$Wt^{0.75}$/15 min after 105 min. Heat production stabilized at 4.80±0.30 cal/$Wt^{0.75}$/ 15 min approximately 150 min after initiation of rewarming. Heat loss increased from an initial level of 0.20±0.02 cal/$Wt^{0.75}$/15 min to 2.68±0.34 cal/$Wt^{0.75}$/15 min after 135 min and stabilized at 2.61±0.25 cal/$Wt^{0.75}$/15 min after 150 min. From an initial value of 7.4±0.2°C, Tbr rose to 35°C after 135 min; Tre rose from 6.8±0.2 to 35°C after 145 min.

Fig. 9. Effects of three i.c.v. injections of 56 µg of 5-HT (C) and 12.5 µg NA (B) on the thermoregulatory dynamics of arousal from hibernation at Ta=6°C. Open circles are heat production; filled circles are heat loss; filled squares are brain temperature; and half-open circles are rectal temperature. Arrows refer to time of drug injections at brain temperatures of 10, 20, and 30°C. All values are $\bar{X} \pm$ S.E.


Brain Temperature
Heat Production
Rectal Temperature
Heat Loss
A Control
B NA
C 5-HT
Calories/Wt·75/15 min
Temperature (°C)
Time (minutes)
0
30
60
90
120
150
2
4
6
10
20
30
40

2. NA

Figure 9B illustrates change of HP, HL and rate of rewarming in the group (n=8) receiving i.c.v. injection of NA. Starting from an initial level of 0.71±.09 cal/$Wt^{0.75}$/15 min HP increased to a peak of 7.31±0.37 cal/$Wt^{0.75}$/15 min after 75 min. HP stabilized at 6.00±0.48 cal/$Wt^{0.75}$/15 min approximately 120 min after initiation of rewarming. Heat loss increased from an initial level of 0.29±0.10 cal/$Wt^{0.75}$/15 min to 3.34±0.21 cal/$Wt^{0.75}$/15 min after 120 min and stabilized at this level. From an initial value of 7.8±0.2°C, Tbr rose to 35°C after 112 min; Tre rose from 7.4±0.3 to 35°C after 118 min.

After the first injection of NA, there was marked acceleration of the rate of increase of HP, HL, and Tbr as compared to the controls. The rate of increase of HP began to exceed that of the control group after 30 min post-disturbance of the animal (7 min after the first injection of NA) and continued until the peak of HP was reached at 75 min. Heat loss began to increase at a rate faster than the control group after 30 min and continued to exceed that of the control until its plateau was reached after 120 min post-disturbance. The rate of increase of Tbr and Tre began to exceed control values after 30 min until maximum temperatures were reached after 120 min.

3. 5-HT

Figure 9C illustrates changes of HP and HL for the group (n=6) receiving i.c.v. injections of 5-HT during rewarming from hibernation. Starting from an initial level of 0.67±0.09 cal/

$Wt^{0.75}$/15 min, HP increased to a peak of 7.18±0.37 cal/$Wt^{0.75}$/15 min after 105 min. Heat production then stabilized at 5.17±0.30 cal/$Wt^{0.75}$/ 15 min after 160 min. Heat loss increased from an initial level of 0.28±0.03 cal/$Wt^{0.75}$/15 min to 3.20±0.22 cal/$Wt^{0.75}$/ 15 min after 135 min, and decreased to 2.61±0.05 cal/$Wt^{0.75}$/15 min after 165 min. From an initial value of 7.8°C ± 0.2 Tbr rose to 33°C after 150 min; Tr rose from 7.0±0.3 to 35°C after 156 min.

After the first injection of 5-HT there was marked suppression in the rate of increase of HP as compared to the controls. This suppression lasted for approximately 40 min after the injection (60 min post-injection). The rate of HL began to exceed that of the controls after 75 min, and continued until 135 min after the initiation of arousal. The rates of increase of both Tbr and Tre were suppressed relative to the controls until 75 min after the initiation of arousal.

Table 1 summarizes the overall changes of HP, HL and rate of rewarming in control, NA and 5-HT injected groups. The 5-HT injected group produced significantly more heat but required the longest time to reach normothermia than did the control or NA injected groups. This was due mainly to the significantly greater HL in the 5-HT injected group as compared to the controls. Significantly less time was required for the NA injected group to reach normothermia than the control group, but the total HP and HL were similar to the control group. The average temperature difference between Tbr and Tre during rewarming was similar between the control and NA injected groups but was significantly less between the 5-HT injected and the control group.

Table 1

Effects of i.c.v. injection of NA and 5-HT on the energetics of disturbed arousal in *Spermophilus richardsonii*

	Total HP cal/$Wt^{0.75}$	Total HL cal/$Wt^{0.75}$	Average Tb-Tr(^{o}C)	Time for Tr to reach 35^{o}C (min)
Control (n=7)	114.30±2.50	31.00±2.70	5.33±0.36	145.30±7.80
NA (n=8)	108.51±3.18	29.68±2.20	5.00±0.34	117.50+3.78 **
5-HT (n=6)	126.27±1.98 **	41.80±1.13 **	3.20±0.44 *	156.00±2.00

* Significantly different from control ($p<0.05$)

** ($p<0.02$)

Values are $\bar{X} \pm$ S.E.

DISCUSSION

Implicit with studies concerned with the thermoregulatory effects of i.c.v. injections of putative neurotransmitters are two basic assumptions: 1) that the injected drug reaches thermoregulatory centers in the rostral hypothalamus by uptake from the ventricular fluid through the ependymal walls of the third ventricle, and 2) that the effects achieved by such injections are resultant from interaction between drug and receptor sites of neurons of thermoregulatory pathways. There is good evidence that drugs injected into the cerebral ventricles are diffused rapidly throughout the ventricular system (Feldberg and Myers, 1964), and subsequently transported across the ependymal walls (Aghajanian *et al.*, 1966). Due to the diffusion of the drugs throughout the entire ventricular system after injection, however, the anatomical specificity of drug action is equivocal. For example, injections of 5-HT or NA may activate serotonergic or noradrenergic pathways associated with the cortex, corpus striatum, hypothalamus, reticular formation, and the hind brain (Myers, 1974). It is not surprising, therefore, that i.c.v. injections of 5-HT and NA may elicit changes in many functions such as drinking and feeding (Myers and Yaksh, 1968), sleeping (Janský and Novotná, 1976), as well as Tb (reviewed by Hellon, 1972). In spite of this anatomical nonspecificity characteristic of the i.c.v. stimulation, however, there is surprising consistency of a drug eliciting a specific effect (Myers and Yaksh, 1968). This is born-out several ways: 1) i.c.v. injection of a specific drug such as 5-HT into a wide variety of

mammals elicits similar thermoregulatory changes provided that the drug concentration, and Ta are constant (Francesconi and Mager, 1976); 2) administration of specific agonists, precursors, or inhibitors of metabolic degradation have effects parallel to those evoked by the drug itself (Myers and Yaksh, 1968), and 3) dose dependent relationships between drug concentration and effect have been demonstrated (Francesconi and Mager, 1976). Historically, i.c.v. injection has proven to be a valuable method in evaluating the central effects of putative neurotransmitters. Within limits, it is still a potentially useful technique in first-hand approximation of the central actions of certain drugs (Myers, 1974).

I. NORMOTHERMIC ANIMALS

A. 5-HT AND HARMALINE

The hypothermia resulting from i.c.v. injections of 5-HT in the Richardson's ground squirrels is consistent with that evoked by i.c.v. injection of 5-HT in many hibernating and non-hibernating species (Cooper *et al.*, 1965; Bligh *et al.*, 1971; Baird *et al.*, 1974; Hlavička, 1976, 1977). Quantitative measurements of the HL and HP components of the thermoregulatory response in the present study have shown, however, that hypothermia may arise either from activation of HL only or from the activation of HL and the suppression of HP. This differential response is dependent on the physiological state of the animal which in turn is determined by the yearly hibernation cycle. During the non-hibernating phase, mild hypothermia (0.40^{o}C) was caused by initial activation of HL (Figs. 2 and 3). This is similar to the

response of the golden hamster to i.c.v. administration of 5-HT (Hlavička, 1976, 1977), where Tb was depressed principally by the activation of HL (there was no prolonged suppression of HP). Injection of 5-HT during the hibernating phase, on the other hand, caused a more marked hypothermia (-1.65^{o}C) due to both activation of HL and suppression of HP (Fig. 3). This type of response was similar to that evoked by i.c.v. injection of 5-HT into rabbits, sheep, and goats (Bligh *et al.*, 1971).

In a complementary study, harmaline, an MAO inhibitor, was centrally injected to observe the effects of manipulation of endogenous 5-HT. This drug is known in rats to cause a 100% increase in brain 5-HT levels without affecting noradrenaline levels (Bruinvels and Sourkes, 1968). Caloric measurements showed that similar to 5-HT injection, differential thermoregulatory response to harmaline injection was also observed and the response was dependent upon the hibernation phase of the animals. During the non-hibernating phase, harmaline caused an increase in Tb (0.80^{o}C) due to the activation of HP with small activation of HL (Fig. 5). During the hibernating phase, however, harmaline caused a suppression of HP with a small decrease in HL resulting in a fall in Tb (0.74^{o}C; Fig. 5). The temperature lowering effect of harmaline in the Richardson's ground squirrel during the hibernating phase at Ta = 5^{o}C is similar to that observed in the rat at Ta = 23^{o}C (Bruinvels and Sourkes, 1968).

That there was differential thermoregulatory response to increased brain 5-HT (by both exogenous administration and enhancement of endogenous 5-HT by harmaline) in ground squirrels during different phases

of the hibernation cycle may be indicative of an endogenous change in central serotonergic pathways incumbent with the yearly hibernating cycle. Furthermore, as the thermolytic action of increased brain 5-HT was more pronounced in the hibernating phase, it is possible that the 5-HT pathways are more active during the hibernating phase. This is in agreement with the observations of Novotná *et al.* (1976), that in the golden hamster kept under natural conditions there was a 14-fold increase in brainstem 5-HT turnover rate during the preparative phase for hibernation which later increased to 24-fold during hibernation. Also, increased brain 5-HT levels have been reported in the hibernating bat (Shaskan, 1969), hedgehog (Uuspaä, 1963), and the golden hamster (Novotná *et al.*, 1975).

Other evidence for changes in 5-HT thermoregulatory pathways during the yearly hibernation cycle has been reported by Janský *et al.* (1973). They found that the decrease in Tb after the peripheral administration of p-CPA was greater in golden hamsters preparing for hibernation than during the late winter. Analysis of brain 5-HT after administration of p-CPA showed that there was 160% increase in 5-HT 30 min after injection and that there was no significant depletion of 5-HT until 6 hrs after injection. The fall in Tb closely paralleled the increase in brain 5-HT. Other experiments conducted by Hlavička (1977) on golden hamsters kept under constant environmental conditions (Ta = 25^{o}C; 12L/12D photoperiod) showed, however, that there was no differential thermoregulatory response to i.c.v. infusions of 5-HT. The total amount of 5-HT infused in their study, however, was relatively small (5 µg) compared to the larger doses which in this study did

elicit significant changes in thermoregulatory responses between non-hibernating and hibernating groups of animals. Hence it may be possible that the dose used by Hlavička was not sufficiently large enough to resolve seasonal differences in responsiveness to exogenous 5-HT. It is also possible that species differences exist since golden hamsters are not seasonal hibernators as are the Richardson's ground squirrels.

To date, there has been much evidence presented indicating the importance of central serotonergic pathways to mammalian hibernation. For example, Spafford and Pengelley (1971) showed that hibernation could be disrupted by the administration of p-CPA or by lesioning the median raphe nucleus; both procedures causing a long-term depression of brain 5-HT. In addition, the ability to hibernate (Spafford and Pengelley, 1970) and the length of hibernation bouts (Janský, 1971) have been correlated with central levels of 5-HT. The functional role of 5-HT in hibernation is, however, still open to speculation. The present study demonstrated that short-term increases in brain 5-HT levels by 5-HT or harmaline injections could elicit differential activation of thermoregulatory pathways during different phases of the hibernation cycle. It is therefore apparent that reorganization of HP and HL pathways may also be part of the repertoire along with other physiological changes during the preparation for hibernation in seasonal hibernators.

Leuke and South (1971) speculated that there is progressive endogenous suppression of hypothalamic thermogenic neurons during the entrance into hibernation. Such a suppression would serve to lower the threshold for cold-stimulated thermogenesis, and consequently cause

an absence of thermogenesis during cold exposure. An updated version of this model (South *et al.*, 1978) requires the existence of two neuronal pools, one inhibitory and the other excitatory, which are functional only during the entrance to and the maintenance of the hibernating state. These neuronal pools are responsible for the maintenance of a depressed but relatively constant Tb during hibernation; an increase in Ta or Tb facilitates the inhibitory neurones and HP is minimalised; a decrease in Ta or Tb disinhibits the thermogenic neurones and HP is increased. The finding that increased brain levels of 5-HT in the Richardson's ground squirrel caused marked suppression of HP only during the hibernating phase may offer a possible neurochemical basis for the models of Leuke and South (1971), and South *et al.* (1978). It is possible that 5-HT may be the neurotransmitter released from the neurones which inhibit the thermogenic neuronal pool. Such a role may also reflect the functional significance of the dramatic increases in brain 5-HT concentration (Uuspaä, 1963; Shaskan, 1969; Novotná *et al.*, (1975) and turnover (Novotná *et al.*, 1975) occurring during late summer and throughout the winter in many hibernating species.

B. NA

There is now very strong evidence supporting the role of NA as a central mediator of thermoregulatory mechanisms. For example, specific hypothalamic sites of action of NA have been mapped (Zeisberger and Brück, 1971, 1976) and the nature of the receptors involved with NA mediated thermoregulatory pathways are known (Dhawan and Dua, 1971; Burks, 1972). Furthermore, single unit activity of thermoresponsive neurones after NA application corresponds well with

changes of Tb observed after central injection of NA (Hori and Nakayama, 1973).

The hyperthermia resulting from i.c.v. injections of NA in the Richardson's ground squirrel is consistent with that found in many non-hibernating (Feldberg and Myers, 1964; Beckman, 1970; Zeisberger and Brück, 1971; Veale and Whishaw, 1976) and hibernating species (Beckman and Satinoff, 1972; Glass and Wang, 1978). Quantitative measurements of the HL and HP components of the thermoregulatory response in the present study have shown, however, that hyperthermia is consequent to the activation of HP rather than the suppression of HL as reported by Cooper *et al.* (1965). Furthermore, the effects of environmental temperature on the thermoregulatory response of Richardson's ground squirrels was not as pronounced as in other studies in which environmental temperature was found to cause both qualitative (Bligh *et al.*, 1971; Avery, 1972) or quantitative (Veale and Whishaw, 1976) changes in thermoregulatory response. In the cold, the NA-induced hyperthermia in the Richardson's ground squirrel was reduced as compared to the response in the warm, due to increased HL in the cold.

Injections of NA at the highest dose (12.5 µg) in the Richardson's ground squirrel during the hibernation phase have shown to elicit greater increases in HP and Tb than during the non-hibernating phase. This differential response is dependent on the physiological state of the animal incumbent with its annual hibernation cycle. During hibernation there is a 35% reduction of hypothalamic NA in the Arctic ground squirrel (Feist and Galster, 1974), and decreases in brain NA

have also been reported during hibernation in the hedgehog (Uuspaä, 1963; Sauerbier and Lemmer, 1977). Also, drastic reductions of brain NA turnover during hibernation have been reported in the thirteen-lined ground squirrel (Draskóczy and Lyman, 1967) and in the hedgehog (Sauerbier and Lemmer, 1977). Furthermore, Faure and Calas (1977) have shown that the *in vitro* uptake of NA by central catecholaminergic fibers is reduced in brain slices taken from the hibernating hedgehog as compared to that in brain slices taken from active hedgehogs. These findings suggest that there is a reduction in the activity of the central noradrenergic neurones during hibernation.

This reduction of central noradrenergic activity during hibernation may be related to the increased responsiveness to i.c.v. injections of NA in the hibernating phase in the Richardson's ground squirrels. Axelrod (1974) has reported hypersensitivity of NA-simulated systems in the pineal gland resulting from reduced input of the innervating noradrenergic tracts. Thus, during prolonged periods of light when noradrenergic input is reduced there is hyper-responsiveness of cyclic AMP generating systems to exogenous NA. Other examples of increased sensitivity of inactive central noradrenergic systems to exogenous NA are reviewed by Dismukes and Daly (1976). Since central noradrenergic pathways have been shown to be involved with HP mechanisms (Zeisberger and Brück, 1971, 1976; Glass and Wang, 1978), the apparently reduced activity of these neurones during the hibernation phase as reported above may result in a hypersensitivity of central HP pathway to exogenous NA. Furthermore, in the Richardson's ground squirrel in the hibernating phase, there is increased thermogenic responsiveness to intravenous infusions of the

β-receptor agonist isoproterenol (Abbotts and Wang, unpublished observations). This hyperresponsiveness may be related to reduced turnover and concentration of NA in peripheral tissues at this time (Uuspaä, 1963; Draskóczy and Lyman, 1967; Sauerbier and Lemmer, 1977).

The demonstration of endogenous seasonal changes in thermoregulatory response after centrally applied NA supports the role of central noradrenergic thermoregulatory pathways in the regulation of hibernation.

II. HIBERNATING ANIMALS

A. ENERGETICS OF DISTURBED AROUSAL

Simultaneous measurements of HP, HL and Tb have shown rewarming from hibernation to be not only an extremely dynamic process, but a surprisingly economical way of raising Tb in the cold. Calculations of the energetics of disturbed arousal in the uninjected control group shown in Table 2 demonstrate the efficiency of the rewarming process. The total HP for rewarming to a Tb of 36°C was 12,274 calories and the total HL for the same period was 3,303 calories. The average weight of the animals was 512 g, and with a specific heat of 0.83 cal/g/°C for animal tissue (Cromer, 1974), the net HP (8,971 cal) should have increased Tb by 21.1°C. However, the actual increase was approximately 29°C (from 7 to 36°C). One explanation for this discrepancy may be that anaerobiosis occurred during rewarming with the oxygen debt being repaid after Tb stabilization. This is unlikely, however, as there was no observed sustained increase in oxygen consumption above the resting level following stabilization of Tb at 36°C (Fig. 9A). The absence of an oxygen debt has also been reported

Table 2

Energetics of disturbed arousal from hibernation in *Spermophilus richardsonii* *

Average Weight (g)	Total HP (cal)	Total HL (cal)	Net Heat (cal)	Calcualted Specific Heat
512±29	12,274±494	3,303±257	8,971±475	0.59 cal/g/^{o}C

* n = 7

Values are $\bar{X}$ ± S.E.

(Hammel *et al.*, 1968; Wang, 1978). Therefore, one must question the validity of using 0.83 cal/g/^{o}C as the specific heat of the animal during rewarming. We measured the cooling of a Richardson's ground squirrel carcass in a calorimeter and found the specific heat of the animal was 0.83 cal/g/^{o}C (Glass and Wang, unpublished). Calculations from HP and HL (Table 2) showed that the specific heat of the animal was 0.59 cal/g/^{o}C. A recalculation based on calorimetric measurements of Hammel *et al.* (1968) in the arousing golden-mantled ground squirrel indicated that the value of the specific heat for their animal was 0.52 cal/g/^{o}C. Moreover, Wang (1978) reported an apparent specific heat of 0.45 cal/g/^{o}C in a Richardson's ground squirrel in an undisturbed arousal. Since only drastic changes of tissue composition could vary the specific heat of a body, it is unlikely that physical modifications were responsible for the lowering of specific heat during arousal. Wang (1978) has suggested that the differential rewarming which occurs during arousal may be responsible for the observed reduction of specific heat; as heat is produced by an inner layer it serves to heat the next outer one, and so on until the outermost layer is warmed. This process would restrict HL, as most of the heat produced would be trapped rather than lost to the environment. Hence, the total HP necessary to raise Tb from hibernating to normothermic levels could be correspondingly reduced. Based on the above experiments the energy saving could range from 30 to 40% due to the reduction of HL.

B. EFFECTS OF 5-HT AND NA ON AROUSAL

1. 5-HT

The initial suppression of HP (from 30 to 60 min post-

disturbance) and overall increased HL caused by i.c.v. injections of 5-HT during arousal is similar to that found in non-hibernating species during normothermia after i.c.v. 5-HT (Bligh *et al.*, 1971; Baird *et al.*, 1974; Hlavička, 1976). Figure 9(c) shows, however, that after 60 min 5-HT failed to cause suppression of HP. Furthermore, the mean rates of HP and net NP during rewarming for the 5-HT injected group were similar to the control group (Table 3), indicating that there was no *overall* reduction in rate of HP by 5-HT. The somewhat greater time required for rewarming (and therefore slower rate of rewarming Table 3) was due to both the initial suppression of HP and the greater overall HL. A slowing of rewarming has also been observed after i.p. injection of 5-HT and 5-hydroxytryptophan (a 5-HT precursor) in arousing red-cheeked ground squirrels (Popova, 1975; Yakimenko and Popova, 1976). As observations by Janský and Novotná (1976) show that there may be penetration of 5-HT through the blood-brain barrier after i.p. injection of 5-HT, it is likely that the slowing of rewarming by i.p. 5-HT was in part centrally mediated.

The smaller average temperature difference between Tre and Tbr in the 5-HT injected group (Table 1) may reflect altered differential rewarming caused by premature decrease of peripheral and posterior vasomotor tone. This may have been due to the action of increased brain 5-HT on vasomotor pathways associated with central HL mechanisms. This argument is strengthened by the observation of increased overall HL in the 5-HT injected group. Furthermore, i.c.v. injection of 5-HT in rabbits, goats, and sheep is known to cause a decrease in peripheral vasomotor tone resulting in increased HL (Bligh *et al.*, 1971).

2. NA

Intracerebroventricular injections of NA, on the other hand, had the effect of causing a significantly larger expenditure of energy and a greater net rate of HP during rewarming than did the control group (Table 3). This resulted in a decreased time required for rewarming (Table 1) and is reflected by a faster rate of rewarming (Table 3). This activation of HP by i.c.v. injection of NA during rewarming is consistent with other studies where central injection of NA activated HP in some non-hibernating mammals such as guinea pigs (Zeisberger and Brück, 1971, 1976), rats (Beckman, 1970), neonatal rabbits (Komaromi *et al.*, 1969); and some hibernating species such as the golden-mantled ground squirrel (Beckman and Satinoff, 1972), the Richardson's ground squirrel (Glass and Wang, this study), and neonatal Columbian ground squirrels (Glass and Wang, 1978). The average temperature difference between Tbr and Tre was not significantly different from the control group (Table 1), indicating that there was no direct influence of increased brain NA levels on the normal vasomotor adjustments which occur during rewarming.

There is a good functional relationship between the actions of 5-HT and NA reported here and their respective levels observed during rewarming. Both Kudryvtseva and Popova (1973) and Feist and Galster (1974) have reported decreases in hypothalamic 5-HT during arousal; the latter authors noting a decrease during the initial phase of rewarming only. If endogenous 5-HT acts to suppress HP and activate HL then it would be expected that there would be relatively low levels of this substance at this time. Feist and Galster (1974) have also reported increased hypothalamic NA during mid-arousal when the rates of

Table 3

Effects of i.c.v. injection of NA and 5-HT on the rates of heat production, rewarming, and excess heat production during disturbed arousal in *Spermophilus richardsonii*

	Rate of HP[#] ($cal/Wt^{0.75}/min$)	Rate of Rewarming ($^{o}C/min$)	Rate of net HP[Δ] ($cal/Wt^{0.75}/min$)
Control (n=7)	0.786±0.037	0.203±0.010	0.5732±0.40
NA (n=8)	0.925±0.025 *	0.255±0.007 **	0.6710±0.027 *
5-HT (n=6)	0.809±0.018	0.179±0.003	0.5420±0.012

* Significantly different from control ($p<0.05$)

** ($p<0.02$)

Values are $\bar{X} \pm$ S.E.

\# Calculated by total $HP/Wt^{0.75}$/time for Tr to reach $35^{o}C$

Δ Calculated by (Total HP - Total HL)/time for Tr to reach $35^{o}C$

increase of Tb and HP are maximal. If endogenous NA activates HP then it would be expected that there would be relatively high levels of this substance at this time. It has also been shown that inhibition of NA synthesis during hibernation prevents complete arousal from hibernation (Feist, 1970).

The observed effects of i.c.v. injection of the biogenic amines, 5-HT and NA on the thermoregulatory dynamics of rewarming in the Richardson's ground squirrel are similar to those evoked in some euthermic hibernators (Beckman and Satinoff, 1972; Baird *et al.*, 1974; Janský and Novotná, 1976; Glass and Wang, 1978) and non-hibernating species (Komaromi *et al*, 1969; Beckman, 1970; Bligh *et al.*, 1971; Zeisberger and Brück, 1971): NA causing activation of HP and 5-HT causing suppression of HP and activation of HL. It is possible, therefore, that these substances mediate thermoregulatory pathways during arousal in much the same manner as during normothermia.

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